WASHINGTON NATURAL HERITAGE PROGRAM

Monitoring Seed Predation of *Sidalcea oregana* (Nutt.) Gray var. *calva* C.L. Hitchcock

Prepared for
U.S. Fish and Wildlife Service
Region 1

Prepared by

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Executive Summary

High levels of seed predation by weevils have been observed for many years on Wenatchee Mountain checker-mallow (*Sidalcea oregana var. calva*), federally listed as endangered under the Endangered Species Act. This project was designed to develop a practical and simplified methodology for monitoring the level of this predation, with the long term objective of being able to discern the effects on weevil populations of different management or naturally occurring treatments. The methodology was refined through three series of monitoring trials, in seven populations of Wenatchee Mountain checker-mallow, in a total of thirteen samples. Two different species of native weevils were observed and tentatively identified as *Macrorhoptis niger* and *Anthonomus sphaeralceae*; other insect species, especially aphids, were abundant and apparently also feeding on the checker-mallow. Seed predation levels by weevils ranged from 25.9 to 59.4 percent; seed loss to other insects, most likely the aphids, ranged up to over 70 percent. A proposed monitoring methodology, based on these trials and evaluating a variety of insect impacts, is presented.

Acknowledgments

This project was funded by the U.S. Fish and Wildlife Service under Section 6 of the Endangered Species Act. We would like to acknowledge the assistance of Lauri Malmquist of the USFS Wenatchee River Ranger District for sharing her knowledge of the sites, assistance with transect locations, and participation in fieldwork. We would also like to acknowledge Tara Goldsmith, whose graduate research on Wenatchee Mountain checker-mallow provided a stepping stone to understanding the ecology of this species. Melanie Gisler provided information about her studies on *Sidalcea hendersonii* and its associated weevils, which supported our learning. Robert Anderson of the Canadian Museum of Nature was an asset in identifying specimens of both weevil species and providing information about their biology. Thanks to Merrill Peterson and Keith Pike for identification of the aphids.

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Introduction

Sidalcea oregana var. *calva* (SIORCA), a plant species endemic to the Wenatchee Mountains in Washington State, was listed as endangered under the federal Endangered Species Act in December, 1999 (Thomas 1999); it is also currently designated as sensitive by Region 6 of the U.S. Forest Service and as endangered by the Washington Natural Heritage Program (WNHP) (2007). SIORCA is one of four species of *Sidalcea* in Washington that have been designated as state endangered, including *S. hirtipes, S. malviflora* ssp. *virgata,* and *S. nelsoniana* (also federally threatened). A fifth *Sidalcea, S. hendersonii,* is increasingly rare in Oregon, but it is locally abundant in Washington. Caplow and Chappell (2005) determined that because of its distribution and abundance in Washington, it does not warrant designation in this state as rare.

The rarity of SIORCA is likely to be at least partially due to human-caused effects, including habitat fragmentation and degradation due to development and ecological changes resulting from fire suppression (Thomas 1999). Other potential threats may include climate change and loss of pollinators, in particular the ground-dwelling bee *Diadasia nigrifrons* (Caplow 2003, Goldsmith 2003, Thomas 1999).

The rarity of SIORCA may also partially be due to insect damage, which may also be related to human-caused ecological changes, particularly those caused by fire suppression. High levels of seed predation by weevils have been observed in *Sidalcea* species, including SIORCA (Gamon 1985, Goldsmith 2003, Gisler and Meinke 1997); Gamon (1987) also noted large numbers of aphids on SIORCA.

Native species of weevil feed on all of these species of *Sidalcea*, and their impact on seed production in SIORCA has been a concern since weevil larvae were observed feeding within fruits in 1987 (Thomas 1999). Goldsmith's work (2003) raised the level of concern about these effects when she observed high levels of seed loss to weevils. She reported that in August 2001 and 2002 only 14% and 17%, respectively, of the seeds appeared to have escaped damage by weevils. These levels of potentially successful seed correspond to those of 14.6% observed by Gisler and Meinke (1997) in *S. nelsoniana*.

Preference by weevils for a rare species over more common congeners has been observed and documented by Gisler and Meinke (1997) on *Sidalcea nelsoniana.* It is possible that the weevils' preference for certain species over others has been a contributor to species rarity. Preference by insects for the seed of another rare species, in this case *Astragalus sinuatus*, over nearby, more common *Astragalus* species has been observed elsewhere in the Wenatchee Mountains (Combs 2005).

The uncertainty in the identities of the weevils that have been observed on SIORCA has illustrated another element of the complexity of questions about their impacts on SIORCA. Dr. Charles O'Brien at Florida A&M University had identified weevils from SIORCA as *Macrorhoptus sidalceae* and an *Anthonomus* species that Goldsmith (2003) did not think was prominent at her research site. Gamon (1987) reported that the weevils as the same site had tentatively been identified as *Macrorhoptus niger.* Robert Anderson from the Canadian Museum of Nature has identified weevils from this site as *M. niger* and *A. sphaeralceae;* he thinks that *M. sidalceae* may be limited to *S. hendersonii*, and that inland weevil species are distinct. He applied the name *M. niger* as a “best fit” determination and is currently involved in DNA bar coding and other molecular analysis with these weevils (personal communication, 6November2006). That more than one species of weevil was involved with SIORCA was not unexpected; both *M. sidalceae* and *A. melancholicus* had been documented as coexisting on *S. hendersonii* in British Columbia (Marshall and Ganders 2001), and both *M. sidalceae* and *M. niger* were reported in populations of *S. campestris* and *S. virgata* in Oregon (Gisler and Meinke (1997).

The symbiosis (used in the broad sense) between *Sidalcea* and *Macrorhoptus* and *Anthonomus* weevils appears to include precise species-specific behavior. Adult weevils chew holes in the carpels and oviposit into the holes, sealing them with a sticky excretion. The larvae develop within the seed, consuming the fruit in patterns unique to each species. After consuming the seed, the larvae metamorphose into the pupa stage within the calyx, often protected by the cover of the wilted corolla. Once fully developed they exit the flower, usually through holes chewed in the calyx.

Gisler reported two distinct feeding behaviors by seed-predating weevils on *Sidalcea hendersonii*. Though she did not observe the fruits being consumed directly, in populations where only *Anthonomus* weevils were present, the larvae consumed the entire fruit, whereas in populations where only *Macrorhoptus* was present, the larvae had tunneled from carpel to carpel and consumed the inside of each. Populations of *Sidalcea* with both weevil species showed both kinds of damage (Melanie Gisler, personal communication, 15August2006).

In addition to the impacts of seed predation by weevils, a substantial amount of seed of SIORCA appears to be lost due to damage caused by other insects, especially aphids. Gamon (1987) noted that the level of impact of the aphids observed on SIORCA had not been documented. We do not know the identity of the aphids, or whether they are native. Spittlebugs (family Cercopidae) were also present in some populations, as well as other insects that we were not able to identify even to family. Pointing out still another dimension of the complexity of the relationship between *Sidalcea* species and insects, parasitism of weevil larvae by wasps was also observed in SIORCA populations (Goldsmith 2003).

Our intention was to develop a practical and cost effective methodology that could be used to evaluate changes in insect impacts over time. Its potential applications could also include evaluation of the effects of management activities, such as tree and brush removal, prescribed burning, and hydrological manipulations.

In order to protect the confidentiality of the SIORCA sites that were monitored, including those on private land, abbreviated names for them will be used throughout this report. Managers and owners are aware of the location of the surveyed populations on their lands.

Methods

This methodology was developed in an iterative process, revising the method in the three successive monitoring series. As the field work progressed, we became more aware of the complexity of the plant-insect interactions involving SIORCA, and more able to distinguish between different types of impacts. As substantial damage by insects other than weevils became apparent, the scope of the methodology was widened to include these other potentially significant impacts.

One objective of this project was to determine the optimum time for monitoring. In 2006, early and late visits were made to the SIORCA population to gain familiarity with the phenology of the plants and the behavior of the insects. The format and types of data to be collected were modified as we understood the interactions of the insects and the *Sidalcea* better.

Sighting records in the WNHP database were reviewed as an indication of potential survey times, and a first, exploratory visit was made to the population on May 12, 2006. At this time, plants were developing but not yet in flower. The draft seed predation monitoring methodology was first conducted on July 18 and 19, 2006 at six sites: Camp Rd, Canyon, Brush Road-left fork, Brush Road-right fork, the Lodge, and the Pond. On August 8 and 9, 2006, sampling was conducted at four sites: the Canyon, the Lodge, the Meadow, and Camp Road. On July 17 and 18, 2007, sampling was conducted at the Canyon, the Lodge, and the Meadow.

Study Sites

All of these populations are documented in the WNHP database, and all have been monitored for population size by the Forest Service (Lauri Malmquist, Leavenworth District botanist, personal communication). In most cases, permanent transects that have previously been established by the Forest Service were used for locating these plots for insect monitoring. Transects are marked at each end with metal fence posts, in order to facilitate relocation for future monitoring.

Population size varies from site to site, and at one site (the Pond) it was necessary to count all individuals within the population due to its small size (as of 2006).

Transect 1: The Camp Road site is located on DNR land and end posts for a 50 meter transect are in place. The plants at this site were observed in 2006 to flower and set seed later than the other populations, most likely due to the presence of a thicker canopy and wetter substrate. The northernmost transect stake is visible from the road.

Transect 2: The Canyon site is located on USFS land and end posts for a 50 meter transect are in place. This area has been selectively thinned by the Forest Service to open the canopy for SIORCA, though the clearing avoided the population of SIORCA itself, and may thereby have resulted in more limited benefit than if the canopy of the population itself was opened.

Transect 3: The Brush Road- left fork site is located on USFS land and end posts for a 50 meter transect are in place, although the population is quite small and there may not be enough plants to collect data on even ten plants in randomly selected plots. The transect is located beneath a forest canopy, and the site is becoming overgrown with snowberry (*Symphoricarpos albus)*. Manual lopping of snowberry has been done within the past few years by the Forest Service (Lauri Malmquist, personal communication) to reduce competition between snowberry and SIORCA.

Transect 4: The Brush Road- right fork site is also located on USFS land. Although the area was large enough to accommodate a 50 meter transect, in 2006 there were not enough plants, when using random plot selection methods, to obtain a substantial dataset for the transect. For this reason, we counted all the individuals that were within 2 meters on either side of the transect. In future years it may be necessary to continue counting all individuals at this site depending on the size of the population. The site is also being overtaken by shrubs (primarily snowberry, among others) which could potentially decrease the number of SIORCA individuals able to survive at this site.

Transect 5: The Lodge site is located on private land and is a relatively large population (300 plants reported in 2002); end posts for a 50 meter transect are in place. The population is located in the meadow below the house. The southern end of the transect is visible from the road. In 2006 there had been some thinning done by the land owners in the area of the population. The machinery flattened the plants (although it probably didn't damage the roots) along the transect, and as a result, another transect was chosen from the southernmost transect stake.

Transect 6: The Pond site is located on DNR land; it is a very small population, not able to accommodate a 50-meter transect, located near an old, relatively small, fenced elk exclosure that can be seen from the road. In 2006 we established a 20-meter transect and collected data on as many plants as possible in randomly selected plots.

Transect 7: The Meadow site is located on DNR land, and is a very large population, with approximately 11,000 plants counted in 2000. There are no transect stakes at this site, so it is necessary to choose an appropriate area, where SIORCA plants are concentrated, and establish a 50-meter transect.

Data collection, July 2006

At each site a tape was laid along the previously established 50-meter transect, if one was present, or along the 20-meter transect at the Pond site. Ten to fifteen plots were randomly selected, out of the possible 100 plots along each 50-meter transect. Plots were one meter square, on either side of the transect (see Figure 1 below). A one-meter frame delineated each plot along the transect. Data was collected from the tallest raceme of the plant closest to the center of the plot. If the panicle was branched, the terminal inflorescence was used. If none of the flowers on a raceme had usable data (i.e. they were dead or not present), the next closest raceme was used. Data for each flower and raceme was recorded on a preliminary datasheet.

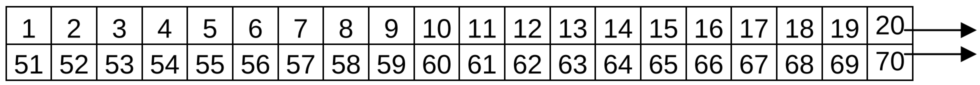

Figure 1. Diagram of plot layout along each transect. Plots on the left side of the transect extend from 1 to 50, and on the right side of the transect from 51 to 100. Only a portion of the transect is illustrated.

If no SIORCA with flowering stems occurred in the selected random plot directly on either side of the transect, then a plant was selected from the adjacent plot, one meter away from the transect (see Figure 2 below). Plants found in these plots were recorded with the same plot number as the plot adjacent to the transect, with the addition of the number of meters the plot was out from the transect (i.e. the starred plot in Figure 2 would be recorded as “7-2”).

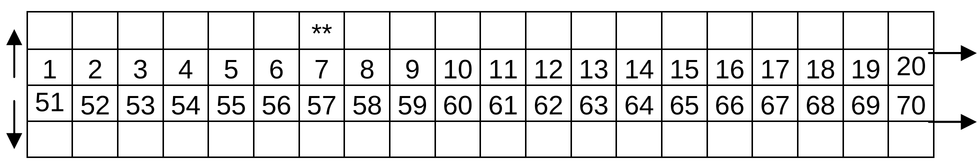

Figure 2. Lateral plots along a transect. If no flowering stems of SIORCA occur within a randomly selected plot, then a plant may be selected from an adjacent plot, moving away from the transect line in one-meter increments.

The following data were gathered on July 18 and 19:

1. Plot #

2. Flower #: flowers were numbered from the bottom of the raceme up, starting with the lowest flower that had developing ovaries. Data were collected from the lower ten flowers, or from as many as were present, up to ten (+ or -). Any obvious chewing/holes in the calyx, caused by insects (presumably weevils) was recorded as +.

3. Carpels (# damaged/ total #): Data were collected on the number of carpels observed that showed oviposition holes, expressed as a percentage of the total number of carpels within each flower (usually 5-9). When total consumption of the fruit was observed, resulting in a completely empty calyx, sometimes with a larva or pupa present, the number of damaged carpels was recorded as X/X. When the site monitoring was complete, the numerical value for X was calculated as the average number of carpels per flower at that location. X for all populations was approximately equal to 7 and this number was used in the calculations.

4. Notes were made on the presence of adult weevils, thrips, aphids, white powder fungus, number of aborted flowers on the raceme, and other insects or damage were recorded.

At transect 4, the Brushy Road-Right Fork site, plots were not used. Because very few plants were present at this site, data was recorded for *all plants* within two meters on either side of the transect. In future monitoring years, the observer must decide to lay out plots or count all plants within two meters, based on the size of population.

At transect 6, the Pond site, we established a permanent transect by starting at the large *Pinus ponderosa* on the site and headed due north for 20 meters. Plots were randomly selected along this transect. There were not enough racemes at this site to randomly choose 15.

Data collection, August 2006

After reviewing data and monitoring methods used during the first visit, we reformatted the datasheets and modified the method of data collection. On August 8 and 9, four of the initially sampled sites were monitored: Transect 1, the Camp Road; Transect 2 , the Canyon; Transect 5, the Lodge; and Transect 7, the Meadow.

Data that were recorded differently in the second visit, for each individual flower, are as follows:

Shriveled or undeveloped carpels were sometimes abundant, and often the entire fruit, and even the calyx, would be shriveled/undeveloped. This damage did not appear due to weevils, and we began specifically recording these shriveled or aborted carpels, independently of whether or not weevil damage was also visible

Empty carpels, appearing externally to be intact, were also observed. We interpreted this as a very different feeding pattern of the weevil larvae. Empty carpels were left behind after the seed had been consumed and the larva had moved on to adjacent seed, and the number of empty carpels within each flower was recorded here. If a larva was present within the seed, actively consuming it, it was scored as an empty carpel. These data were difficult to obtain, and required that each carpel be removed from the fruit and examined from beneath.

Aborted or undeveloped flowers, appearing small, brown, and withered, were sometimes abundant, generally located below the intact flowers. Generally, no carpel development was observed, and we interpreted this as usually due to other insect activity, possibly failure of pollination or herbivory by other insects that were observed, including thrips and spittlebugs. We recorded these aborted flowers separately, as a count per inflorescence. We did not count all the flowers within an inflorescence, and so cannot represent these aborted flowers as a proportion of the entire potential of the plants.

Withered flowers often occurred within the fruit bearing portion of the inflorescences. If all the carpels in a flower (and often the calyx as well) were withered, we included these carpels in the total count of potential seeds, and scored them as having been lost to insects, likely aphids. If withered carpels showed evidence of oviposition, we included them in the measure of weevil impacts as well. Accordingly, weevil impacts, and aphid impacts could add up to more than 100 percent. This allowed us to evaluate weevil effects and effects of other insects independently.

A variety of organisms where observed that may have been impacting the SIORCA. Presence or absence (+ or -) of the following was noted: adult weevils, thrips, a white powdery fungus, spittle bugs, and other insects or damage present. Photographs in Appendix II show the appearance of various insects that were observed on SIORCA.

Weevil identification: Specimens of weevils found on SIORCA were preserved in ethanol and sent to Robert Anderson at the Canadian Museum of Nature for identification.

2007 Monitoring Trials

In 2007 the methodology was again revised and conducted at three sites: the Meadow on July 17, and the Canyon and the Lodge on July 18. Additional modifications to the methodology and data sheet were made and are incorporated into the final monitoring protocol, included in Appendix I.

Weevil identification: In 2007 additional specimens of weevils found on SIORCA, as well as on *S. hirtipes* and *S. nelsoniana*, in populations elsewhere in Washington, were collected and preserved in ethanol and sent to Robert Anderson at the Canadian Museum of Nature for identification.

Results

Observations of Insect Behavior

As we observed the interaction between SIORCA and numerous insects, the complexity of these interactions became evident. The following sections include our observations on these interactions.

Seed predation: Initially we were unable to distinguish between the species of weevils feeding on SIORCA, and it was difficult to separate out the effects of these two different insects. Weevils were found in every population of SIORCA that we examined, as well as other *Sidalcea* species. Typically they were found in mature, open flowers, and the most frequent pose was head down towards the bottom of the flower (see Figure 3). We interpreted this pose to indicate a female that was chewing into the top of the *Sidalcea* ovaries in preparation for ovipositing. The insects were frequently observed mating, and one was observed ovipositing into a hole that had been prepared.

In 2006 we observed that one species of weevil (thought to be *Anthonomus sphaeralceae*) on SIORCA laid eggs and progressed through instars earlier in the season, consuming the entire schizocarp while in the larval stage, leaving an empty calyx occupied by a larva or pupa. During the visit later in the season, a smaller, darker adult weevil with a much shorter rostrum was more abundant (thought to be *Macrorhoptus niger*). The larvae appeared to tunnel through the fruit, consuming the seed from the inside, but leaving an empty carpel behind. The observations that two feeding strategies were employed corresponded with observations by Gisler on *Sidalcea hendersonii* (Melanie Gisler, personal communication, 15August2006).

Often these carpels appeared healthy from the outside, but when they were removed from the maturing fruit and examined carefully, a weevil larva could be seen feeding inside. Incorrectly perceiving these empty seed coats as viable would overstate the likely reproductive output of the plants and understate the effect of the weevils.

The direct evidence of seed predation by weevils was abundant. In addition to the presence of the adult insects themselves, small holes chewed into the top of developing *Sidalcea* ovaries were common, and microscopic examination of the developing seeds below these holes never failed to show an egg or a growing larva within the seed tissue. In some cases the adjacent carpels would remain intact, and could potentially develop into a viable seed. This was uncommon, and almost always, some of the larvae appeared to tunnel from carpel to carpel, consuming each in turn, but leaving an intact seed coat. The larvae were not visible unless the carpel was removed and examined from below. Other larva appeared to consume the whole fruit, and removing the withered corolla would expose an empty calyx, or sometimes a fat larva or pupa. We expect that the likelihood of successful development of any seed within a fruit penetrated by even one weevil egg is low.

Aborted flowers: We observed many flowers that failed to develop, or that started to mature carpels and then withered entirely. Many of these were located below the developing flowers, and appeared to have no carpel development. We interpreted these to have failed to develop because of inadequate pollination, or because the vascular tissue supplying them was damaged by insects, early in the development of the flowers. Aphids, leaf hoppers, and spittle bugs were commonly observed on SIORCA, and are potential sources of this damage. Our impression, which needs to be confirmed, was that spittlebugs and leafhoppers were more abundant early in the season, and that aphids increased later.

Other flowers appeared to have begun to develop carpels, and some even showed evidence of weevil oviposition, before the flower and the developing fruit withered completely. The weevil larvae, if they were present in these fruits, would have apparently also dried up in the process. Because of the abundance of aphids, in the genus *Uroleucon*, at this stage of plant development, we think that these insects are the likely source of this damage, tapping the vascular tissue supplying the flowers before development is complete (See Figure 4).

Figure 3. Photograph of an adult weevil in a SIORCA flower

Figure 4. Photograph of aphids on SIORCA

Measures of Insect Damage

The three summary values that most explicitly express the levels of insect damage are 1) the percent of all carpels produced calculated to have been consumed by weevils, 2) the percent of all carpels produced that appear to have been made inviable by other insects, most likely aphids, and 3) the percent of all carpels produced that appear to have the potential to develop viable seed. These are summarized in Table 1, by population and date of sampling.

Table 1. Summary of data from monitoring insect damage on SIORCA, 2006 and 2007. In 2006, damage to carpels was attributed to either weevils or other insects, and the total for damaged and apparently viable seed equaled 100 percent. In 2007, different kinds of damage to carpels were scored independently and equaled greater than 100 percent, because a carpel could be damaged by both weevils and other insects.

population	date	total carpels	carpel loss to weevils (%)	carpel failure due to insects other than weevils (%)	apparently viable seed by external exam (%)	apparently viable seed by internal exam (%)	apparently viable seed in flowers with no weevil oviposition (%)
Canyon	July 18, 2006	660	45.5	9.5	45.0	-	6.4
Camp Road	July 18, 2006	638	43.6	8.8	47.6	-	5.8
Brush Road - left	July 19, 2006	820	32.7	30.6	36.7	-	15.0
Brush Road - right	July 19, 2006	478	25.9	35.1	38.9	-	22.4
Lodge	July 19, 2006	894	50.3	10.5	39.3	-	0.9
Pond	July 19, 2006	289	51.2	0.0	48.8	-	27.0
Canyon	August 8, 2006	525	47.6	15.2	-	4.2	4.6
Camp Road	August 9, 2006	547	55.9	33.3	-	11.0	4.8
Meadow	August 9, 2006	386	59.1	40.4	-	1.0	0.3
Lodge	August 9, 2006	532	59.4	36.8	-	3.8	0.4
Canyon	July 17, 2007	365	38.9	21.4	41.1	-	13.7
Meadow	July 18, 2007	252	41.3	40.5	23.4	-	7.5
Lodge	July 18, 2007	567	27.2	74.8	11.3	-	4.2

Table 1 shows that loss of carpels due to weevils is quite high (fourth column), ranging from 25.9 to 59.4 percent. However, this value was based only on carpels that were either observed to have holes made during oviposition, or observed to have been eaten by weevils. The likely level of impact is much higher, based on observations made later in the season when carpels were examined internally (seventh column). Assuming that all or nearly all the carpels in a flower with any weevil oviposition will likely be consumed, which our observations suggest, a fairly close approximation of ultimate seed output may be made by counting only those sound-appearing carpels in flowers with no signs of oviposition (eighth column). Based on this assumption and our observations, the range of viable seed production ranges from 0.3 to 27 percent.

Tables 2, 3, and 4 show the percentages of carpel conditions and standard deviations in each of the thirteen samples, giving percent lost to weevils (Table 2), percent lost to other insects (Table 3), and percent likely to develop into seed (Table 4).

Table 5 summarizes the condition of all 6,701 carpels that we examined. It shows that 46 percent of all carpels showed evidence of weevil damage, 28 percent were lost to other insect activity, and 32 percent of the carpels examined appeared sound. The percentages of different damages exceed 100 percent, because in the later samples the damages due to weevils and other insects were calculated independently; some carpels showed both kinds of damage. Assuming that all seeds in a flower would be lost once weevil oviposition occurred, only 8 percent of the carpels we examined were likely to produce viable seed.

Table 2. Percent carpel loss to weevils and standard deviation for each SIORCA population sampled

population	date	carpel loss to weevils (%)	standard deviation (%)
Canyon	July 18, 2006	45.5	2.3
Camp Road	July 18, 2006	43.6	2.2
Brush Road - left	July 19, 2006	32.7	2.8
Brush Road - right	July 19, 2006	25.9	2.6
Lodge	July 19, 2006	50.3	2.1
Pond	July 19, 2006	51.2	3
Canyon	August 8, 2006	47.6	2
Camp Road	August 9, 2006	55.9	3
Meadow	August 9, 2006	59.1	3.5
Lodge	August 9, 2006	59.4	2.7
Canyon	July 17, 2007	38.9	2.5
Meadow	July 18, 2007	41.3	2.6
Lodge	July 18, 2007	27.2	1.8

Table 3. Percent carpel loss to insects other than weevils and standard deviation for each population of SIORCA sampled

population	date	carpel failure due to insects other than weevils (%)	standard deviation (%)
Canyon	July 18, 2006	9.5	0
Camp Road	July 18, 2006	8.8	0
Brush Road - left	July 19, 2006	30.6	0.4
Brush Road - right	July 19, 2006	35.1	3.5
Lodge	July 19, 2006	10.5	2.2
Pond	July 19, 2006	0.0	n.d.
Canyon	August 8, 2006	15.2	2.1
Camp Road	August 9, 2006	33.3	2.9
Meadow	August 9, 2006	40.4	3.2
Lodge	August 9, 2006	36.8	2.9
Canyon	July 17, 2007	21.4	2.5
Meadow	July 18, 2007	40.5	3.2
Lodge	July 18, 2007	74.8	2.9

Table 4. Percent of apparently viable seed and standard deviation for each SIORCA population sampled. In this table, only those carpels in flowers with no evidence of weevil oviposition were scored as likely to be viable.

population	date	Likely viable seed in flowers with no weevil oviposition (%)	standard deviation (%)
Canyon	July 18, 2006	6.4	3.6
Camp Road	July 18, 2006	5.8	3.8
Brush Road - left	July 19, 2006	15.0	3.3
Brush Road - right	July 19, 2006	22.4	3.5
Lodge	July 19, 2006	0.9	2.4
Pond	July 19, 2006	27.0	0.5
Canyon	August 8, 2006	4.6	3.3
Camp Road	August 9, 2006	4.8	0.6
Meadow	August 9, 2006	0.3	0.3
Lodge	August 9, 2006	0.4	0.5
Canyon	July 17, 2007	13.7	3.3
Meadow	July 18, 2007	7.5	3
Lodge	July 18, 2007	4.2	2.5

Table 5. Summary of SIORCA seed production, including effects by weevils and other insects

	count	**percentage of total carpels evaluated**
Total carpels evaluated	6,701	100
Carpels with apparent weevil damage	3,068	46
Carpels with apparent damage from other insects, likely aphids	1,850	28
Carpels that appear sound	2,135	32
Carpels that appear sound in flowers with no weevil oviposition	545	8

Discussion

The motivation for monitoring insect impacts on SIORCA grew out of a need to inform decisions about potential management treatments, such as prescribed fire and canopy reduction. But the complexity of the interactions between SIORCA and insects points out the need to understand the ecology of these plants and the insects that feed on them before levels of impact can be accurately estimated. The summary in Table 1 documents the high level of impacts to SIORCA by insects and includes several values that point out the complexity of this ecology.

The level of seed predation by weevils is quite high, but estimating very precisely how high it may be depends on interpretation of the evidence. The differences between the values in the sixth column in Table 1 - apparently viable seed based on external exam - and the eighth column - apparently viable seed based on the assumption that only seeds in flowers with no evidence of weevil oviposition are likely to develop to maturity - is extreme.

The lowest estimate of damage assumes that only carpels showing direct damage – the hole from oviposition – will be consumed. If a flower includes seven carpels, and one is seen to have been penetrated by a weevil, the adjacent carpels retain the potential to develop into viable seeds. Six good seeds are possible. A predation level of 14.3 percent for that flower is calculated.

The highest estimate assumes that if any evidence of weevil oviposition occurs within a flower, then all the seed in that flower will be consumed. Larvae either tunnel from carpel to carpel, leaving empty seed coats, or consume the entire fruit. A predation level of 100% for that flower is calculated. The latter estimate is likely closer to the actual levels of predation.

In addition to the need to interpret evidence of weevil activity, the high level of impact due to other insects, likely aphids (see the fifth column in Table 1) illustrates that while weevil predation accounted for the largest portion of the insect damage observed, it was only one type of insect impact to SIORCA.

Differences in phenology also add to the challenges of accurate monitoring. Based on two years of sampling, there appears to be fairly wide variation in insect levels, depending upon when they are measured, within a year. Timing of flower development and insect activity also seems to vary year to year. Another unknown in the calculations is that a few flowers continue to develop after monitoring, and these may be more or less vulnerable to seed predation than the earlier developing seeds.

The variation in the numbers of undamaged flowers during the two sample dates in 2006 was most likely due to the unique phenologies of the two species of weevils. Based on our observations in these SIORCA populations, it appears that *Anthonomus* adults lay eggs earlier in the season than *Macrorhoptus.* Our early sampling date may have largely accounted for damage done by *Anthonomus* larvae, while the later sampling date included damage done by both weevil species.

In 2007, mid-July was nearly too late, as many of the flowers had withered. Because of wide variation during the season, tying the monitoring to the phenology of the *Sidalcea* may be critical if the results are used to compare level of effects year to year.

Overall, our sampling revealed that only between 8 and 32 percent of SIORCA seed is likely to succeed to maturity; the lesser of these values is most likely close to the actual level. It is apparent that weevil predation and other insect damage are having a deleterious effect on seed production by *Sidalcea oregana* var. *calva*, and these impacts are likely affecting the reproductive success of the species.

Recommendations

These rounds of sampling were exploratory in nature, and the method was revised with each application. A series of consistent monitorings should now be conducted, including later in the season, and, if possible, before and after treatments on the sites there the species occurs. Annual monitoring of SIORCA and insect populations could provide useful information for comparing SIORCA population data and weevil predation levels over time.

Conducting prescribed burns at one of the SIORCA populations would be an ideal opportunity to use this methodology to measure the effects of this management treatment. Both the plants and the insects evolved together under conditions of a higher fire frequency. Because *Sidalcea oregana* var. *calva* is historically located in areas where late-season, low-intensity burns occurred frequently (Caplow 2003, Goldsmith 2005), it could be predicted that the plant species would respond positively to fire, as it would reduce its competition with encroaching shrubs and increase plant vigor, as well as possibly keeping populations of weevil and other harmful insects in check.

It would be informative to clarify the species of weevils are present, their origins, and the relative abundance of each, particularly if the species respond differently to environmental changes, such as those caused by fire or canopy removal. Perhaps even more important will be to determine the identity of the aphids and other insects feeding on SIORCA. While entomologists assume that these weevils are native, we do not know the identity of the aphids, or whether they are native as well. Knowing the origin of these organisms and their response to management treatments will be central to determining appropriate management strategies.

Pesticide use for weevil control is discouraged as it could potentially affect the plant's specialized pollinator, the ground dwelling bee *Diadasia nigrifrons* (Caplow 2003, Goldsmith 2005) along with a species of native parasitic wasp, which uses the weevils as its host species (M. Gisler, personal communication). The weevils themselves are also apparently native species, and possibly as rare as the plants upon which they depend.

On-going Monitoring

The method as presented in Appendix I can be conducted by one person in less than a day at each site: two people working as a team are more efficient, since one person can examine the plants and insects while the other records data. Two people should be able to conduct the monitoring considerably faster and visit two or more of the sites in a single day. We recommend repeating the protocol annually at several sites, in order to build up an understanding of annual variation in level if insect infestation, and to get a more clear understanding of the annual variation in phenology. Trial runs early and late in the season could yield more understanding of the different behavior of the two weevil species. A single late season monitoring may give the best estimate of the final level of insect damage, if it is conducted before seed dispersal begins. Using the method to detect insect responses to management actions, including tree or shrub removal and controlled burning, is its intended purpose.

Literature Cited

Caplow, F. 2003. Report on the status of *Sidalcea oregana* (Nutt.) Gray var. *calva* C.L. Hitchcock. Natural Heritage Report 2003-1, Washington State Dept. of Natural Resources, Olympia.

Caplow, F. and C. Chappell. 2005. Washington Status of *Sidalcea hendersonii* (Henderson's checkermallow). Natural Heritage Report 2005-04, Washington State Department of Natural Resources, Olympia.

Combs, J.K. 2005. *Atragalus sinuatus* (Piper), a Washington State Endangered Plant Species: Pre-dispersal Seed Predation, Seedling Recruitment and Interactions with *Bromus tectorum* L. M.S. Thesis, University of Washington, College of Forest Resources.

Gisler, Melanie Marshall and Rhoda M. Love. 2005. Henderson's checkermallow: The natural, botanical and conservation history of a rare estuarine species. *Kalmiopsis,* 12: 1-8.

Gisler, S.D., and R.J. Meinke. 1997. Reproductive attrition by pre-dispersal seed predation in *Sidalcea nelsoniana* (Malvaceae): implications for the recovery of a threatened species. T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke, and M.V. Wilson, editors. Conservation and Management of Native Plants and Fungi: proceedings from a conference of the Native Plant Society of Oregon, pp. 56-61.

Goldsmith, T. J. 2003. Factors affecting population growth and persistence of *Sidalcea oregana* (Nutt.) Gray var. *calva* C.L. Hitchcock (Malvaceae), a taxon endemic to

Chelan County, Washington. Unpublished masters thesis, College of Forest Resources, University of Washington.

Huseby, P. 2000. A preliminary study of seed germination in *Sidalcea oregana* var. *calva*. Undergraduate research paper, unpublished, University of Washington.

Marshall, M. and F.R. Ganders. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plant. *American Journal of Botany,* 88: 1437-1443.

Sleeper, E.L. 1957. Notes on the genus *Macrorhoptus* Leconte (Coleoptera: Curculionidae: Anthonominae). The Ohio Journal of Science, 57 (2): 70-74.

Thomas, T. 1999. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for *Sidalcea oregana* var. *calva* (Wenatchee Mountains Checker-Mallow). Federal Register, vol. 64, num. 245: 71680-71697, 22, Dec 1999. Retrieved from the Federal Register Online via GPO access.

Washington Natural Heritage Program (WNHP). 2007. List of plants tracked by the Washington Natural Heritage Program. Available at http://www.dnr.wa.gov/nhp/refdesk/lists/plantrnk.html

Zimmerman, T.G. and S.H. Reichard. 2005. Factors Affecting Persistence of Wenatchee Mountains Checker-Mallow: An Exploratory Look at a Rare Endemic. *Northwest Science* 79 (2&3): 172-178.

Appendix I: Proposed *Sidalcea oregana* var. *calva* (SIORCA) Seed Predation Monitoring Methodology

This methodology is intended to provide a standardized and efficient method for monitoring insect predation on SIORCA. It is a protocol that might be used to evaluate changes in seed output in response to management activities, such as prescribed fire and brush or tree removal, and in response to natural ecological changes like wildland fire. Data sheets for field data collection and for summarizing site data are provided at the end of this paper.

This methodology is designed to generate two types of information:

1. A measure of likely viable seed production

2. A quantitative comparison of the effects of different types of insect activity, especially seed predation by weevils (*Anthonomus* and *Macrorhoptus* species) and loss of flowers because of aphids or other insects.

Materials needed: 50 meter tape, 1 meter PVC frame(s), clipboard, datasheets, hand lens. Permission must be obtained by the managers or owners of any property before entering, and activities involving SIORCA should be conducted with the knowledge and approval of the SIORCA recovery or technical team. This team includes staff from the U.S. Fish and Wildlife Service, the U.S. Forest Service, the Washington Natural Heritage and Natural Area Programs, and the University of Washington.

Transect layout

Sampling is generally conducted along 50 meter transects located at SIORCA sites; where available we have used population monitoring transects marked by shoulder-high metal stakes at either end. In other areas transects may be laid out to cross through SIORCA populations and modified to fit the characteristics of the site.

Plot layout and selection

One square meter plots are arranged as shown in Figure 1 below, below, on either side of the 50 meter transect. Plots are numbered 1-50 on one side, and 51-100 on the other.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	→
51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	→

Figure 1. Plot arrangement along a 50-meter transect. Only a portion of the transect is illustrated.

10 to15 plots are randomly selected for monitoring. If no flowering SIORCA plants with flowering stems occur within the plot adjacent to the transect, then the plot can be moved away from the transect one or two meters, one meter at a time, to record an adequate number of plants. Plants found in these plots are recorded with the same plot number as the plot adjacent to the transect, with the addition of the number of meters the plot is out from the transect (i.e. the starred plot in Figure 2 would be recorded as “7-2”).   If the random plot numbers are arranged in order prior to data collection, and the plots are sampled from one end of the transect to the other, it will minimize the amount of walking back and forth, and potential trampling, along the transect.

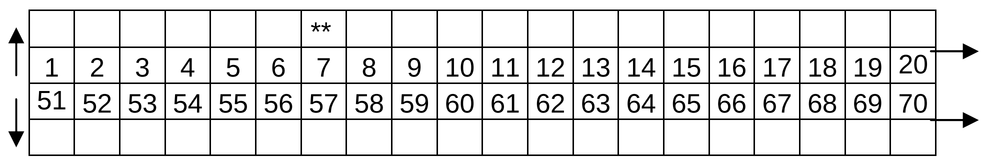

Figure 2. Lateral plots along a transect. If no flowering stems of SIORCA occur within a randomly selected plot, then a plant may be selected from an adjacent plot, moving away from the transect line in one-meter increments.

Sampling and recording data

An insect damage field data sheet is provided in this appendix. Field data are recorded on these sheets; a summary by population is calculated on the insect damage site summary form.

The flowering raceme closest to the center of the frame is chosen from each plot. If the inflorescence is branched, the terminal raceme is scored. If all the flowers on a raceme are dead or undeveloped for some reason, resulting in no recordable seed predation data, then the recordable raceme closest to the center of the plot is sampled.

Data recorded for each plant/raceme: As indicated on the field data sheet, the number of aborted or undeveloped flowers and the presence or absence of adult weevils, aphids, thrips, and other insect damage are noted for each plant examined. The plot number is also recorded.

On each raceme there are usually a few flowers that failed to develop or were obviously aborted at some point due to insect damage to connective tissue, or for other reasons. These flowers appear small, shriveled and brown, with no recordable data inside them. The total number of these undeveloped flowers is recorded for each raceme.

Data recorded or each flower: Flowers with developing carpels are numbered from the bottom of the raceme upward. Up to ten flowers are examined in each raceme. In each flower that has developing fruit, whether calyx damage is present (usually a hole chewed in the side) is noted. Counts are recorded of the total number of carpels (usually 5-9), the number of carpels showing evidence of weevil egg laying (a distinctive round hole in the top of the carpel), the number of carpels that are withered or undeveloped, and the number of carpels that appear sound.

In some flowers, the sum of the number of weevil damaged carpels, the number of shriveled/undeveloped carpels, and the number of apparently good seeds may exceed the total number of carpels recorded. A carpel may show both weevil damage in the form of an ovipositon hole, and shriveling due to the action of other insects. Each of these impacts is calculated separately.

When the calyx is empty (with or without larva present, but without shriveled or aborted carpels) the fruit is presumed to have been eaten by weevils; the carpel number and the number of carpels consumed are recorded as X.   At the end of the sampling the numerical value for X is calculated as the average number of carpels per plant in the population being examined.

Site Summaries:
For each site, several insect damage datasheets are generated; the data from each datasheet is compiled on one line on the site summary form, and the totals for the population are added in the columns. Percentages can be calculated and placed in the summary column in the second section of the site summary sheet. The site summary form gives a one page review of the status of insect damage at each SIORCA population.

Insect Damage Field Data Sheet	**Site name:**	**Transect #**
Plant species:		**Date:**
Surveyors:		

If a mature calyx is completely empty, the assumption is made that all carpels have been consumed by weevil larvae. The carpel # and # with weevil damage are recorded as "X" and are later calculated as the average number of carpels/flower in the population. Damaged carpels plus the # that appear sound may exceed the total # as one carpel may exhibit both types of damage.

Data recorded for each plant/raceme						**Data recorded for each flower (numbered from the bottom of the raceme upwards)**						
								Number of carpels				
Plot #	# of aborted flowers below developed flowers	adult weevils present (+ or -)	aphids present (+ or -)	spittle bugs present (+ or -)	thrips present (+ or -)	**Flr. #**	Calyx damage (+ or -)	total #	# with weevil damage	# present but shriveled or un-developed	# that appear sound	Comments

Insect Damage Site Summary	**Site Name:**
Plant species:	**Date:**
Surveyors:	

*The total number of carpels and the number of weevil damaged carpels both include the number calculated to have been totally consumed, based on the average number of carpels observed per flower in the population, in flowers with empty calyces.

	Of _____ **plants** examined:					Of ________ **flowers** examined:			Of ____________ **carpels** examined:			
	number of aborted flowers below developed flowers	Number of plants with…				number with with insect-damaged calyx	Number of flowers containing fruits that have been…		Total #	# with weevil damage*	# present but shrivelled or un-developed	# that appear sound
Data Sheet Number		adult weevils	aphids	spittle bugs	thrips		undamaged by weevils	totally consumed (calyx empty)				
Total												

	Average number of aborted or undeveloped flowers per inflorescence
	Percentage of plants with adult weevils
	Percentage of plants with weevil larvae or pupae
	Percentage of plants with aphids
	Percentage of plants with spittle bugs
	Percentage of plants with thrips
	Percentage of calyces with insect damage
	Percentage of flowers containing fruits with no weevil damage
	Percentage of flowers with carpels/seeds totally consumed by larvae, calyx empty
	Percentage of carpels with weevil damage (including those totally consumed leaving an empty calyx)
	Percentage of carpels that are shriveled or un-developed
	Percentage of carpels that appear sound